Japanese Patent No. 2884466

Registration Date: February 12, 1999

Application No. 348476/1993

Filing Date: December 27, 1993

Laid Open No. JP 7-187989A

Laid Open: July 25, 1995

Patentee: NAGASE & CO., LTD.

Title of the Invention:

Perilla extract, method for production thereof, and whitening cosmetics containing the same

Claims:

1. A method for producing Perilla extract which comprises subjecting a Perilla family plant to an extraction treatment with a lower alcohol having 10-30 vol%, adding water thereto, removing the precipitate thus produced, concentrating the extracted solution thus obtained, then adding a lower alcohol thereto until the alcohol concentration becomes 90 vol% or higher, and removing the precipitate thus produced to obtain a liquid component again.

2. A method for producing Perilla extract which comprises subjecting to an extraction treatment with a lower alcohol having 10-30 vol%, concentrating the extracted solution thus obtained, then adding water thereto

until the alcohol concentration becomes 10 vol% or lower, removing the precipitate thus produced, concentrating the extracted solution thus obtained, then adding a lower alcohol until the alcohol concentration is 90 vol% or higher, removing the precipitate thus produced to obtain a liquid component again, and adding activated carbon thereto to remove remaining impurities.

3. The method of Claim 1 or 2, wherein the Perilla family plant is Perilla frutescens (L.) Britton var. acuta Kudro, Perilla frutescens (L.) Britton var. acuta Kudo formaviridis Makino, Perilla frutescens (L.) Britton var. crispa (Thunb.) Decne, or Rosmarinus officinalis.

4. A melanin production inhibitor comprising a Perilla extract obtained by subjecting a Perilla family plant to an extraction treatment with a lower alcohol having a water content of 10-30 vol%, adding water thereto, removing the precipitate thus produced, concentrating the extracted solution thus obtained, then adding a lower alcohol thereto until the alcohol concentration becomes 90 vol% or higher, and removing the precipitate thus produced.

5. A melanin production inhibitor comprising a Perilla extract obtained by subjecting a Perilla family plant to an extraction treatment with a lower alcohol having a water content of 10 to 30 vol%, concentrating the extracted

solution thus obtained, then adding water thereto until the alcohol concentration is 10 vol% or lower, removing the precipitate thus produced, concentrating the extracted solution thus obtained, then adding a lower alcohol thereto until the alcohol concentration becomes 90 vol% or higher, removing the precipitate thus produced to obtain a liquid component again, and then adding activated carbon thereto to remove remaining impurities.

6. The melanin production inhibitor of Claim 4 or 5, wherein the Perilla family plant is Perilla frutescens (L.) Britton var. acuta Kudo, Perilla frutescens (L.) Britton var. acuta Kudo formaviridis Makino, Perilla frutescens (L.) Britton var. crispa (Thunb.) Decne or Rosmarinus officinalis.

7. A whitening cosmetic comprising a Perilla extract obtained by subjecting a Perilla family plant to an extraction treatment with a lower alcohol having a water content of 10-30 vol%, adding water thereto, removing the precipitate thus produced, concentrating the extracted solution thus obtained, then adding a lower alcohol until the alcohol concentration becomes 90 vol% or higher, and removing the precipitate thus produced.

8. A whitening cosmetic comprising a Perilla extract obtained by subjecting a Perilla family plant to an

extraction treatment with a lower alcohol having 10 to 30 vol%, concentrating the extracted solution thus obtained, then adding water until the alcohol concentration becomes 10 vol% or lower, removing the precipitate thus produced, concentrating the extracted solution thus obtained, then adding a lower alcohol until the alcohol concentration is 90 vol% or higher, removing the precipitate thus produced to obtain a liquid component again, and then adding activated carbon thereto to remove remaining impurities.

9. The whitening cosmetic of Claim 7 or 8, wherein the Perilla family plant is Perilla frutescens (L.) Britton var. acuta Kudo, Perilla frutescens (L.) Britton var. acuta Kudo formaviridis Makino, Perilla frutescens (L.) Britton var. crispa (Thunb.) Decne, or Rosmarinus officinalis.

[Detailed Description of the Invention]

[0001]

[Industrial Application Field]

The present invention relates to a method for producing a Perilla extract which is effective in whitening the skin or preventing or eliminating sunburn or skin pigmentation such as chloasma and freckle. More particularly, the present invention is concerned with a method for producing a Perilla extract containing rosemaric acid in a high concentration but containing little pigment

component peculiar to Perilla and employable suitably for whitening cosmetics. The present invention is further concerned with using the Perilla extract as a melanin production inhibitor effective in whitening the skin or preventing or eliminating sunburn or skin pigmentation such as chloasma and freckle, as well as a whitening cosmetic containing the melanin production inhibitor.

[0002]

[Prior Art]

Methods for obtaining an extract from a Perilla family plant with use of hot water or carbon dioxide in liquid phase have heretofore been studied (JP 62-65660A and JP 60-120957A). However, these methods mainly aim at obtaining the extract without spoiling the pigment component or fragrance component. Since this extract contains a large amount of pigment peculiar to Perilla, as well as sugar and tannin, it presents a very deep yellowish brown, greenish brown, or reddish brown and has a peculiar smell. If this extract is mixed into a cosmetic or an external medicine for the skin, thee will arise problems in pharmaceutical preparation such as coloration and unstabilization of an emulsion system. As a result, its dosage form and content are fairly restricted.

[0003]

It has already been known that rosemaric acid exhibits pharmacological effects such as anti-inflammation effect and anti-allergy effect as an effective component of Perilla family plants, including Perilla frutescens (L.) Britton var. acuta Kudo, Perilla frutescens (L.) Britton var. acuta Kudo formaviridis Makino, Perilla frutescens (L.) Britton var crispa (Thunb.) Decne, Rosmarinus officinalis as a kind of herb, peppermint, Kawamidori, and daisy.

These plants have long been utilized as highly safe folk medicines. Thus, an extract obtained by extracting any of the above Perilla family plants with hot water for example contains rosemaric acid. However, even if an attempt is made to utilize the drug efficacy of rosemaric acid by mixing such an extract into a cosmetic or an external medicine for skin, there arise the foregoing pharmaceutical problems such as coloration caused by admixture components and unstabilization of an emulsion system. Therefore, the amount of the extract used is limited and, in the application to a cosmetic or an external medicine for the skin, it is difficult to use the extract at a concentration high enough to exhibit the drug efficacy of rosemaric acid.

[0004]

With such a situation as background, there has been

proposed a method for extracting and purifying rosemaric acid in as a pure form as possible from a Perilla family plant and applying it to a cosmetic (JP 63-162611A). However, the proposed method includes such fairly complicated operations as an extraction step which uses a dangerous solvent, e.g., ethyl ether, and a column chromatographic purification step, and requires the use of special apparatus. Thus, the method in question is not advantageous as an extraction and purification method from a Perilla family plant.

[0005]

Pigmentation of the skin such as sunburn or chloasma or freckle results from the phenomenon that cell melanocyte present in an epidermal cell diffuses to adjacent cells. Various medicines are known which suppress or directly inhibit the production of enzyme tyrosinase playing an important role in the formation of melanin at the melanocyte. Kojic acid and arbutin are known as typical medicines heretofore used. Melanin is produced by an enzymatic or non-enzymatic oxidizing action from dopa or dopaquinone resulting from the action of enzyme tyrosinase. Also known are various medicines which inhibit the said process and thereby suppress the formation of melanin. As typical examples of such medicines are mentioned ascorbic

acid and hydroquinone. However, when toxicity against humans, sensuous influence on the skin, and stability are taken into account, the above melanin production inhibitors are not always satisfactory as cosmetic materials. Thus, the development of a highly stable and stable melanin production inhibitor is desired.

[0006]

[Problems to be Solved by the Invention]

In view of the above-mentioned problems in pharmaceutical preparation which occur in case of mixing the conventional Perilla extract into a cosmetic or an external medicine for skin and problems which occur in extraction and purification, the present invention aims at providing a Perilla extract containing in a high concentration of such efficacious components as caffeic acid, perillaldehyde, and rosemaric acid contained in a Perilla family plant, e.g., Perilla frutescens (L.) Britton var. acuta Kudo, Perilla frutescens (L.) Britton var. acuta Kudo formaviridis Makino, Perilla frutescens (L.) Britton var. crispa (Thunb.) Decne, or Rosmarinus officinalis, the Perilla extract containing little deep yellowish, greenish, or reddish brown pigment peculiar to Perilla, as well as a novel industrially advantageous method for producing the Perilla extract. It is also an object of the present

invention to provide a Perilla extract highly safe against humans and employable as an extremely excellent melanin production inhibitor.

[0007]

[Means for Solving the Problems]

For achieving the above-mentioned objects the present inventors have established a Perilla extract producing method comprising the following steps. The present inventors were the first to find out that the said Perilla extract had an enzyme tyrosinase production inhibiting action and was employable as a highly saft and stable melanin production inhibitor, and in this way completed the present invention.

[0008]

More specifically, according to the present invention, a Perilla family plant is subjected to an extraction treatment with a lower alcohol having a water content of 10-30 vol%, the extracted solution thus obtained is concentrated to 10 vol% or lower, then water is added thereto to an alcohol concentration of 10 vol% or lower, the precipitate thus produced is removed, the extracted solution thus obtained is again concentrated to 10 vol% or lower, then a lower alcohol is added to an alcohol concentration of 90 vol% or higher, the precipitate thus

produced is removed, a liquid component is again recovered to obtain an extracted solution, further, activated carbon is added and stirred to remove remaining impurities, whereby the desired Perilla extract can be obtained. This Perilla extract is highly safe, employable as a stable melanin production inhibitor, and can be mixed into a whitening cosmetic.

[0009]

Still more specifically, as the Perilla family plant, there may be used Perilla frutescens (L.) Britton var. acuta Kudo, Perilla frutescens (L.) Britton var. acuta Kudo formaviridis Makino, Perilla frutescens (L.) Britton var. crispa (Thunb.) Decne, or Rosmarinus officinalis. Leaves and stems are mentioned as plant portions which containmuch efficacious components exhibiting the melanin production inhibiting action. It is optional whether leaves and stems are to be used as they are after collection or are to be used after subsequent drying such as sun drying. In point of extraction efficiency it is preferable that leaves and stems be used in a cut state into small pieces. Dried leaves of Perilla frutescens (L.) Britton var. crispa (Thunb.) Decne are available commercially as crude drug and may be used in the invention.

[0010]

As to the amount of the lower alcohol having a water content of 10-30 vol% used for component extraction, i.e., the amount of a hydrous alcohol, an amount thereof which permits leaves and stems to be immersed therein suffices, but is preferably 5 to 15 times as large as the amount of the Perilla family plant used. In case of using an undried plant, it is desirable that the concentration of alcohol in the extraction solvent used be set rather high, taking the water content in the plant into account. As examples of the lower alcohol are mentioned methanol, ethanol, n-propanol, isopropanol, and t-butanol, with ethanol and methanol being preferred. If the water content of the extraction solvent used is outside the range of 10-30 vol%, unintended components and pigments will be mixed into the extracted solution.

[0011]

The extracting operation may be performed at room temperature, but is preferably performed by heating under reflux cooling, whereby the melanin production inhibiting component is extracted rapidly and efficiently. The extraction pressure may be atmospheric pressure and the extraction time is preferably 2 to 48 hours though it differs depending on the extraction temperature. After the extracting operation, an insoluble residue is removed by

filtration. If the extracting operation is repeated also for the residue, it is possible to increase the yield. The extracted solution thus obtained is concentrated to 10 vol% of lower of the volume of the extracted solution, then water is added to an alcohol concentration of 10 vol% or lower, preferably followed by standing for 15 hours or more at 4-10°C, and the precipitate thus produced is removed by filtration for example. The extracted solution thus obtained is again concentrated to 10 vol% or lower, then a lower alcohol is added to an alcohol concentration of 90 vol% or higher, preferably followed by standing for 15 hours or more at 4-10°C, then the precipitate thus produced is removed by filtration for example, and a liquid component is again recovered as an extracted solution. Then, 0.5-2 wt% of activated carbon is added to the extracted solution, followed by agitation, and remaining impurities are removed to obtain the desired Perilla extract. It has become clear by a qualitative test that the Perilla extract thus obtained contains such substances as caffeic acid, perillaldehyde, and rosemaric acid. The present inventors were the first to find out that the Perilla extract exhibits a melanin production inhibiting action.

[0012]

[Example 1] Producing Perilla (Prilla frutescens (L.) Britton var. acuta Kudo) Extract

600 L of 80 vol% ethanol (water content: 20 vol%) was added to 50 Kg of Perilla leaves (Uchida Wakan), followed by standing at 40°C for 24 hours to carry out dip extraction. The extracted solution thus obtained was filtered, then 600 L of 80 vol% ethanol was further added to remaining Perilla leaves and the same operation as above was performed. Extracted solutions obtained twice were combined together to afford 1097 L of an extracted solution (a primary extracted solution). The primary extracted solution was concentrated to 50L at 50°C under reduced pressure, then 100 L of water was added, followed by standing at 10°C for 24 hours. The resulting insoluble precipitate was removed by filtration, then 0.75 wt% of activated carbon was added and, after agitation for 1 hour, was removed by filtration. The residue after the filtration was washed with 3 L of 90 vol% ethanol and a combined total of 102 L of a Perilla extract was obtained. According to a tyrosinase activity measuring test in Example 2, this extracted solution of Perilla is a fraction exhibiting a melanin production inhibiting action. (In the tyrosinase activity measuring test, this extracted solution from Perilla is used as "Perilla extract").

[0013]

[Example 2] Tyrosinase Activity Effect

Mouse-derived melanoma cell B 16 strains were inoculated into animal culture flasks each containing 30 ml of Eagle' s MEM culture medium which contains 150 cm^2 of 10% fetal bovine serum so that the cell density thereof became 2.5 x 104 cells/cm^2. After culture for 24 hours at 37°C under 5% CO_2, the substances shown in Table 1 below were added into the above flasks so as to give the working concentrations shown in the same table, and the culture was continued for 3 days under the same conditions.

[0014]

Thereafter, the cultured product was treated with a 0.25% trypsin solution, then the cells were collected and washed with two 10 ml portions of a PBS (-) buffer solution, then were suspended in 2 ml of a 0.1M phosphoric acid buffer solution (pH 6.8) containing 0.1% Triton X100. After ultrasonic treatment, centrifugal separation was performed at 12000 rpm for 20 minutes to afford a supernatant liquid as a tyrosinase fraction.

[0015]

0.5 ml of the tyrosinase fraction and 0.5 ml of a phosphoric acid buffer solution (pH 6.8) containing 0.05% of L-DOPA were mixed together and absorbance of light at

475 nm was measured at room temperature with the lapse of time, and tyrosinase activity was determined from an initial speed thereof. A total protein content in the tyrosinase fraction was determined in accordance with a manual of Bio-Rad Proten Assay (Bio-Rad Co.).

Table 1 Tyrosinase production inhibiting activity

Measuring Agent	Working Concentration μg/ml	Tyrosinase Activity ΔOD_{475}/min./mg Protein	Percent Inhibition %	Melanin Content $OD_{400}/1 \times 10^6$cells	Percent Melanin Production %
Control		5.748×10^{-2}	0	1.0320	100
Arbutin	10	1.399×10^{-3}	75.7	0.6210	60.2
	30	6.662×10^{-3}	88.4	0.5050	48.9
Kojic acid	200	9.358×10^{-4}	83.7	0.9025	87.5
	400	2.380×10^{-4}	95.9	0.6440	62.4
Perilla Extract	3.0	3.618×10^{-3}	37.1	0.5035	48.8
	6.0	2.221×10^{-2}	61.4	0.6470	38.4

[0017]

From the tyrosinase activity measuring test of Table 1 it is seen that the Perilla extract obtained according to the present invention exhibits an action of inhibiting the production of enzyle tyrosinase which plays an important role in the formation of melanin at melanocyte and that the

formation of melanin is suppressed by the said action. That action is unique in point of exhibiting a high percent tyrosinase inhibition and a low percent melanin production at an extremely low concentration in comparison with arbutin and kojic acid. An extracted solution obtained using Rosmarinus officinalis in the same manner as above also exhibited a melanin production inhibiting action.

[0018]

[Example 3] Cleansing Cream

The Perilla extract obtained in Example 1 is used as a melanin production inhibitor in the following formulation (total 100 wt%) of a face washing cream:

Component A	wt%
myristic acid	14.0
stearic acid	12.0
lauric acid	3.5
oleyl alcohol	1.5
coconut oil fatty acid amide propylbetaine	10.5

Component B	wt %
concentrated glycerin	18.0
potassium hydroxide	7.0
purified water	balance
preservative (p-hydroxybenzoate)	proper amount

Componnet C	wt%
Perilla extract	0.5
fragrance	0.2

Component A is heat-dissolved and held at 80°C. Separately, component B heat-dissolved at 80°C is added to component A, followed by intimate mixing. Cooling is performed under agitation and component C is added at 50°C. In this way a face washing cream was obained.

[0019]

[Example 4] Skin Lotion

The Perilla extract obtained in Example 1 is used as a melanin production inhibitor in the following formulation (total 100 wt%) of skin lotion.

Component	wt%
purified water	balance
concentrated glycerin	4.0
sorbitol solution (70 wt% aqueous solution)	4.0
citric acid (pH controller)	proper amount
sodium citrate	0.3
polyoxyethylene cured castor oil	0.5
ethanol	15.0
Perilla extract	1.0
fragrance	0.05

All the components were agitated and mixed at room

temperature into a homogeneous solution and the pH of the solution was adjusted to 5.5 to afford skin lotion.

[0020]

[Example 5] Milky Lotion

The Perilla extract obtained in example 1 is used as a melanin production inhibitor in the following formulation (total 100 wt%) of milky lotion.

Component A	wt%
purified water	balance
sugar fatty acid ester (S-160, a product of Daiichi Kogyo Seiyaku Co.)	1.0
concentrated glycerin	6.0
preservative (p-hydroxybenzoate)	proper amount
carboxy vinyl polymer	0.06
potassium hydroxide	0.028

Component B	wt%
olive oil	4.0
jojoba oil	4.0
myristyl lactate	2.0
self-emulsifying glycerin monostearate	1.5
lipophilic glycerin monostearate	1.5

Component C	wt%
Perilla extract	0.5
fragrance	0.2

Component A is heat-dissolved and is held at 80°C. Separately, component B is added to heat-dissolved component A, followed by intimate mixing. Cooling is performed under agitation and component C is added at 50°C. Milky lotion was obtained in this way.

[0021]

[Example 6] Cream

Component A	wt%
purified water	balance
concentrated glycerin	6.0
1,3-butylene glycol	2.0
preservative (p-hydroxybenzoate)	proper amount
carboxy vinyl polymer	0.22
potassium hydroxide	0.15

Component B	wt%
squalene	7.0
olive oil	10.0
jojoba oil	5.0
self-emulsifying glycerin	

monostearate	1.5
lipophilic glycerin monostearate	1.5
polsolvate 60	1.8
sorbitol monostearate	0.5

Component C	wt%
Perilla extract	1.5
fragrance	0.2

Component A is heat-dissolved and is held at 80°C. Separately, component B heat-dissolved at 80°C is added to component A, followed by intimate mixing. Cooling is performed under stirring and component C is added at 50°C. Cream was obtained in this way.

[0022]

Thus, the Perilla extract obtained from a Perilla family plant is mixed as a whitening component into cosmetics such as cleansing cream, skin lotion, milky lotion, and cream. When the cosmetics were applied to humans, a sensuous influence thereof on the skin was very good.

[0023]

[Effect of the Invention]

It is known that the components of Perilla family plants exhibit anti-inflammation action, anti-allergy

action, antispastic action, and analgesic action. The components in quesiton have long been used as folk medicines. However, it has so far been not known that they are useful in whitening the skin, preventing or eliminating skin pigmentation such as sunburn and chloasma or freckle, and further in suppressing the formation of melanin. The Perilla extract obtained by the present invention, which contains caffeic acid, perillaldehyde, and rosemaric acid, exhibits an action of suppressing the production of enzyme tyrosinase which plays an important role in the formation of pigment melanin at the cell melanocyte present in the epidermal cell. From the results of the tyrosinase activity measuring test of Table 1 it has become clear that the formation of melanin is suppressed by the said action. Thus, the Perilla extract is not only preferable from the standpoint of safety but also is extremely useful as a melanin production inhibitor. Further, cosmetics containing the Perilla extract have an excellent whitening effect.